THE METHODOLOGY OF EMPLOYING BACT. PERFRINGENS TOXIN IN THE HISTOCHEMICAL INVESTIGATION OF COLLAGEN

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THE METHODOLOGY OF EMPLOYING BACT. PERFRINGENS TOXIN IN THE HISTOCHEMICAL INVESTIGATION OF COLLAGEN

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It has recently been demostrated that collagen is a multi-phase, at least a two-phase, system. The collagen complex is composed of soluble procollagen and insoluble collastromin, which are, in turn, complex protein-polysaccharide groups. These two phases are firmly interconnected in the collagenic fibers of normal connective tissue.

It has also been demonstrated that procollagen greatly determines the structure and tinctorial properties of collagen. The typical inter-band distances of 2, 9, and 11 A on the roentgenogram, the cross-striation under electron microscopy, the intensive red color stain with picrofuchsin, characteristics so typical of collagen, are caused by procollagen and are not intrinsic to collastromin (A. A. Tustanovskiy, A. L. Zaydes, A. L. Zaydes, G. V. Orlovskaya, A. N. Mikhaylov).

In a biochemical experiment it was determined that Bact. perfringens toxin specifically breaks down the separate procollagen fraction, the degree of splitting depending on the mode and density of procollagen packing in the various formations (A. A. Tustanovskiy; A. A. Tustanovskiy, K. I. Strachitskiy, K. F. Fifarova). Native collagen, however, is not split by collagenase but is subject to the action of the latter only in a denatured state (Gersh and Catchpole, A. A. Tustanovskiy, Aikat and Dibbl).

Under pathological conditions of connective tissue, the internal bonds in the collagen complex are weakened and the procollagen can be displaced and subjected to the

splitting action of collagenase - the K-toxin (G. V. Orlovskaya).

These characteristics of the pathologically modified, disorganized collagen complex are put at the basis of the histochemical analysis of collagen by means of the comparison of the picrofuchsin specific stain before and after incubation of test tissue slices in a collagenase solution (G. V. Orlovskaya; A. I. Strukov, A. A. Tustanovskiy, G. V. Orlovskaya, N. T. Raykhlin).

In practical work the majority of investigators have to deal as a rule with dry toxin Bact. perfringens prepared from filtrates of microbe cultures. Owing to this, other histolytic substances (lipases, hyaluronidases, esterases and others) are present in the toxin in addition to the specific enzyme - collagenase, which selectively breaks down procollagen; the former are capable of acting on the most diverse tissue components in one or another degree, which gives no grounds to think that any of these enzymes whatever is collagenase (E. Pirs). Since this circumstance can to a large extent veil and even distort the findings, the method is undoubtedly diminished in value. The use of complex enzyme groups of such type, however, makes it possible to obtain interesting results (E. Pirs). When studying the characteristics of collagen, therefore, the investigator must constantly take into account the secondary action of the K-toxin employed and try in every way to exclude the latter's influence in the results obtained.

When investigating vascular connective tissue in the case of rheumatism, we employed, for analysis of collagen characteristics, a dry purified Bact. perfringens toxin prepared by the Institute of Epidemiology and Microbiology imeni Gamaleys, Academy of Medical Sciences USSR.

A special examination of dry toxin was made to determine the best conditions for the action of the specific collagenase enzyme and the exclusion of the undesirable influence of other enzymes.

The walls of large vessels of cadavers of patients who died from rheumatism served as the investigation material, the object of K-toxin action. The vessels of cadavers of virtually healthy people who had died suddenly of trauma and the vessels of umbilical cords of newborn infants were used as the control. After fixation in cold acetone, six pieces were sealed in one paraffin block: three pieces from the cadavers of patients and three control. On the microscope slide two slices 8μ in thickness were pasted side by side; one of them was subjected to enzyme treatment.

In this manner maximum parity of conditions was achieved with respect to incubation periods, the mode of subsequent treatment and staining, thickness of slices and

so forth.

Experiments were conducted in 16 series with each series varied in incubation period (from 6 to 24 hours) and concentration of K-toxin solution (from 0.1 mg. x 1 ml. to 2 mg. x 1 ml.). The K-toxin solution was prepared in a phosphate buffer with pH = 6.8. Incubation of slices in the solution was carried out in a thermostat at a temperature of about 37°; subsequent staining with picrofuchsin after Van Gieson.

Considerable fading, yellowing or complete disappearance of the red picrofuchsin staining of pathologically changed collagen after K-toxin treatment, in the case of the absence or negligible fading of the staining of normal collagen after treatment in the same conditions, are considered a manifestation of the specific collagenase effect of K-toxin which breaks down procollagen in the disorganized collagenic complex.

The change of the red staining both of pathologically modified as well as of normal collagenic fibers, sometimes to the point of completely removing the stain in all slice structures, indicates gross disintegration both of collagen as well as of other tissue components, which is apparently connected with the splitting action of the complex of secondary enzymes present in the toxin solution (see table).

The findings of our experiments cited in the table show a definite tendency to the selective action of collagenase in impaired collagenic tissue. Clearly expressed at the same time is the effect that incubation periods and solution concentrations have on the K-toxin's selective collagenase action and the solution's secondary activity. The toxin's non-specific action, masking and distorting results, are intensified with increased solution concentration and especially with a lengthening of the incubation periods.

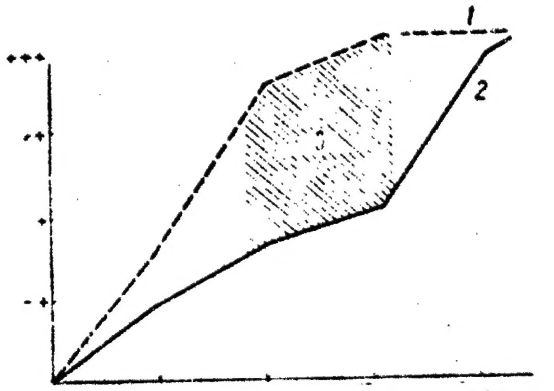
Conditions (a definite incubation period at a definite concentration) can, however, be distinguished, which we call the zone of the collagenase optimum; within the limits of this zone the specific action of K-toxin on impaired tissue is most pronounced. The zone of the collagenase optimum has a more or less broad interval between the expressed action of collagenase and the action of non-specific enzymes, which is an essential premise to the authenticity of histochemical analysis. Only those findings obtained as a result of K-toxin action in the conditions of the collagenase optimum zone can, consequently, indicate the disorganization of the collagenic complex.

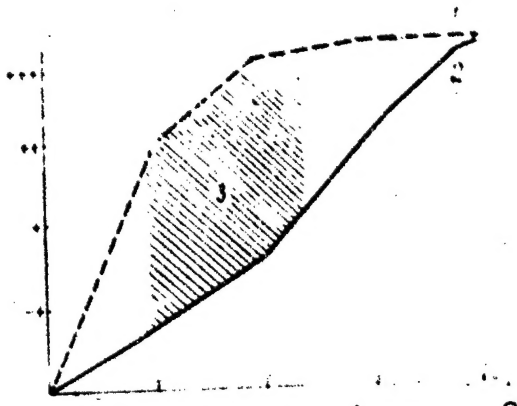
Changes of normal and disorganized collagen under various conditions of K-toxin treatment

Solution concentration	Incubation period in hours	Collagen of normal vessel walls			Collagen of vessel walls of patients who died from rheumatism		
		umbilical cord of the newborn	coronary artery	aorta	coronary artery acute rheumatism	coronary artery relapsing rheumatism	Aorta
0.1 mg. x 1 ml.	6	−+	−	−	−	−+	−+
	12	−+	−	−	−	+	+
	18	−+	−+	−+	+	++	++
	24	+	−+	−+	++	+++	++−
0.3 mg. x 1 ml.	6	−+	−+	−+	+	+	+
	12	+	−+	−+	+	+++	+++
	18	++	+	+	+++	+++	+++
	24	++	++	+−	+++	+++	+++
1 mg. x 1 ml.	6	+	+−	+−	++	+++	+++
	12	++	++	++	+++	+++	+++
	18	+++	+++	+++	+++	+++	+++
	24	+++	+++	+++	+++	+++	+++
2 ml. x 1 ml.	6	−+	++	++	+++	+++	+++
	12	++	++	++	+++	+++	+++
	18	+++	+++	+++	+++	+++	+++
	24	+++	+++	+++	+++	+++	+++

Conventional symbols: minus (−) - absence of change in stain after incubation in K-toxin; minus plus (− +) - thinning and negligible fading of staining of single fibers; plus (+) - distinct fading and disappearance of stain in the majority of fibers; two pluses (+ +) - almost complete disappearance of stain: feebly stained single fibers; three pluses (+ + +) - complete disappearance of stain.

In the figure shown it is evident that for a given series of Bact. perfringens toxin the collagenase optimum zone lies in the area of solution concentrations 0.1 - 0.3 mg. x 1 ml. of buffer solution and incubation periods of 12 to 18 hours (see graphs).





Summary graphs of collagenase optimum zone depending on incubation periods and solution concentrations of K-toxin.
1 - curve of the effect in disorganized collagen;
2 - curve of the effect in normal collagen;
3 - zone of collagenase optimum (the remaining symbols are the same as in the table).

It should be noted that the collagenase optimum zone can fluctuate within certain limits with respect to its width (the size of the interval between the toxin's specific and non-specific effect) as well as the conditions (solution concentrations, action periods) that depend on the virulence of the stain of bacteria from which the toxin is prepared, the periods of toxin preparation and degree of its purification. To get clear results when working with K-toxin, it is therefore necessary in advance to determine the collagenase optimum zone for the given series of Bact. perfringens toxin by checking the effect of specific and non-specific enzymes on normal tissue and collagenic tissue known to be impaired, in various conditions.

The method described makes it possible to determine rapidly and with sufficient clarity the collagenase optimum zone of K-toxin.

Bibliography

Orlovskaya, G. V., Arkh. pat., 1958, No. 10, page 48.

Orlovskaya, G. V., Strukov, A. I., Tustanovskiy, A. A., V kn. Gistokhimicheskiye metody v normal'noy i patologicheskoy morfologii (Histochemical Methods in Normal and Pathological Morphology). M., 1958, page 134.

Pirs, E., Gistokhimiya teoreticheskaya i prikladnaya (Theoretical and Applied Histochemistry). M., 1956, pages 285, 293, 294.

Raykhlin, N. T., V kn. Gistokhimicheskiye metody v normal'noy i patologicheskoy morfologii (Histochemical Methods in Normal and Pathological Morphology). M., 1958, page 145.

Strukov, A. I., Orlovskaya, G. V., Ann. Rheumat. Dis., 1957, Vol. 16, page 307.

Tustanovskiy, A. A., Prokollageny i nekotoryye ikh svoystva (Procollagens and Some of Their Properties). Diss. dokt. M., 1951.

Tustanovskiy, A. A., Strachitskiy, K. I., Fifarova, K. F. V kn. Vopr. Med. Khimii (Problems in Medical Chemistry). M., 1951, Vol. 3, page 176.

Tustanovskiy, A. A., Zaydes, A. L., Orlovskiy, G. V. et al. Dokl. AN SSSR, 1954, Vol. 97, No. 1, page 121.

Aikat, B., Dibbl, J. H., quoted by E. Pirs (1956), page 293.

Gersh, I., Catchpole, H. R., Am. J. Anat., 1949, Vol. 85, page 457.

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